

NOG-0009

#9/B (NE) 58
3/3/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Patent Application of

Hideshi FUJIWAKE

Group Art Unit: 1637

Serial No. 09/828,211

Examiner: J. Tung

Filed: April 9, 2001

For: METHOD OF DETECTING MUTATION
IN BASE SEQUENCE OF NUCLEIC
ACID

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RESPONSE TO FINAL OFFICE ACTION 1637/2900

Assistant Commissioner of Patents
Washington, DC 20231

Sir:

This is a full and timely response to the non-final Official Action mailed October 25, 2002. Reexamination and reconsideration in light of the following remarks are courteously requested.

Claims 1 to 6 are currently pending for the Examiner's consideration. In the Office Action, the Examiner rejected claims 1 to 6 under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,795,976 ("Oefner") in view of U.S. Patent No. 6,268,146 ("Schultz"). These rejections are respectfully traversed.

To summarize the arguments that will follow, claim 1 is directed to a method of detecting mutations in the base sequence of a nucleic acid where the method enables discrimination and inspection of mutations in the base sequence of a plurality of inspected cites in a single nucleic

*the amendment
had been entered
in paper #4
J. Tung 3/11/2003*

acid, and in a single analysis by way of ion pair chromatography. The Oefner and Schultz references, singularly or combined, fail to teach or suggest the present invention as defined in the claims.

Oefner discloses a method for detecting a single nucleic acid heteroduplex molecule using HPLC. Oefner uses oligonucleotides to hybridize a nucleic acid fragment. However, Oefner is not directed to the use of *"a plurality of types of oligonucleotides that are labeled to be discriminable from each other"* as recited in claim 1. The reason for this is that Oefner's method is only suitable for the detection of single base-pair mismatches in a nucleic acid, or consecutive base-pair mismatches that are flanked by matching base pairs. In other words, the Oefner method is similar to those disclosed in the background section of the present application, and suffers from the same inadequacies, namely, the inability to detect and adequately analyze a plurality of sites that may have base pair mismatches.

From the above discussion, it is clear that Oefner also fails to teach or suggest the feature in claim 1 where *"a plurality of inspected sites to be subjected to inspection of mutation in the base sequence [are hybridized] with a plurality of types of oligonucleotides..."* Oefner repeatedly teaches that the method disclosed therein is suitable for detecting a single base pair mismatch in a nucleic acid of up

to 2,000 bp, but fails to teach or suggest the detection of a plurality of mismatches, which would require a plurality of oligonucleotides.

The present invention overcomes the deficiencies of the Oefner reference, and the similar deficiencies discussed in the background section of the present invention, by using ion pair chromatography. Using this technology, it is possible to mix a plurality of oligonucleotides that are individually complementary to at least one normal (non-mutational) base sequence of one of a plurality of sites to be inspected in a single nucleic acid strand or fragment, as claimed in claim 1. Because the temperature in the ion pair chromatograph is set "at a temperature at which there is a difference in stability between the hetero- and homoduplexes included in the duplexes for analyzing the object of analysis," as claimed in claim 1, the detector can discriminate the oligonucleotides, and detect the presence of one or more mutations in the base sequence of the nucleic acid.

The Examiner concedes that Oefner is deficient with respect to the claimed step of using a plurality of labeled oligonucleotide probes to detect multiple mismatches on a single stranded target nucleic acid. The Examiner applies Schultz as allegedly providing teachings that compensate for the deficiencies of Oefner, and cites Schultz's disclosure of the use of a plurality of oligonucleotides in performing a

multiplex assay. See column 15, lines 1 to 63. However, Schultz fails to teach or suggest the use of ion pair chromatography to accomplish the detection of the presence of one or more nucleic acid target sequences in a sample, as recited in the present claims.

Schultz includes two embodiments, namely:

1) a first embodiment directed to a method for determining the presence or absence of a predetermined nucleic acid target sequence in a nucleic acid sample (col. 5, line 42 to col. 6, line 9; col. 11, line 38 to col. 12, line 9); and

2) a second embodiment directed to a method for determining the presence or absence of a mismatch at the 3' terminal nucleotide in a nucleic acid hybrid, which is formed by hybridization of a predetermined nucleic acid target sequence with a nucleic acid probe (col. 12, lines 10 to 51. This second embodiment is concerned with the degree of hybridization or the degree of complementarity.

The second embodiment of the Schultz disclosure is most pertinent to the present invention, and will be discussed first. According to Schultz, a sample to be assayed is admixed with one or more nucleic acid probes to form a hybridization composition. The 3' terminal region of the probes includes an identifier nucleotide and hybridizes with partial or total complementarity to the nucleic acid target sequence of the sample. The hybridization composition

hybridized with total complementarity is a homo-duplex and the hybridization composition hybridized with partial complementarity is a hetero-duplex. Both the homo- and hetero-duplexes are formed according to the Schultz method.

Next, the treated sample is admixed with a depolymerizing enzyme to release one or more nucleotides from the 3' terminus of a hybridized nucleic acid probe. In the enzyme reaction, a nucleotide is released when the hybridization at the 3' terminus is completely matched (i.e., forming a homo-duplex), while a nucleotide is not released when the hybridization at the 3' terminus is mismatched (i.e., forming a hetero-duplex). Depending on the type of enzyme, a nucleotide is released at the mismatched 3' terminus (col. 14, lines 44 to 54). Finally, the released identifier nucleotides are analyzed to obtain an analytical output by absorbance spectroscopy or the like.

In the first embodiment of the Schultz method, however, the presence or absence of a mismatch can only be determined at the 3' terminus region. There is very little discrimination between a strand having a match and a strand having a mismatch when a single mismatch is ten to twelve residues from the 3' terminal nucleotide position (col. 12, lines 35 to 46). In contrast, the presently claimed invention includes the detection step of employing an ion pair chromatograph comprising a reversed phase column serving as a

separation column and a detector capable of discriminating and detecting the labeled oligonucleotides, and setting the separation column at a temperature at which there is a difference in stability between the hetero- and homo-duplexes includes in the duplex strands for analyzing the object of analysis (claim 1). This method is not dependent on the position of a mismatch, that is, any position of a mismatch upstream from the terminus of the labeled oligonucleotide probes.

A further difference between the Schultz method of the second embodiment and the present invention lies in the substance that is being analyzed. According to Schultz, the identifier nucleotides, which are released by the enzyme reaction, are analyzed. In contrast, the present invention involves the analysis of compositions of nucleic acids or nucleic acid fragments themselves, hybridized with labeled oligonucleotides.

Regarding the first embodiment of the Schultz disclosure, Schultz discloses various methods, including fluorescence HPLC and mass spectrometry, where release of indicator nucleotides is quantitatively determined by a single peak at a greater level than background noise, and such a release indicates the presence of a target nucleic acid. However, such a peak is due to the presence of an oligonucleotide that is complementary to a mutated base sequence, and not to a normal

base sequence of a target site as recited in pending claim 1.

Further, Schultz does not teach or suggest the step of discriminating homo- and hetero-duplexes from one another by setting a separation column at a specific temperature, as Schultz does not teach or suggest the formation of both homo- and hetero-duplexes, since the oligonucleotides used in the Schultz method are specific to mutated target nucleic acid sequences, and not to normal target sequences. In contrast, the present invention involves the use of ion pair chromatography methods to discriminate homo- and heteroduplexes from one another, where such complexes are created by hybridizing a nucleic acid with a plurality of oligonucleotides that are complementary to normal (non-mutated) nucleic acid sequences at inspected sites.

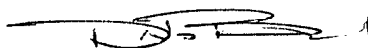
"To establish prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)." M.P.E.P. § 2143.03. Accord. M.P.E.P. § 706.02(j). Because the features discussed above in the claims are neither taught nor suggested by the combination of Oefner and the first or second embodiments of the Schultz disclosure, it is respectfully requested that the rejections of claims 1 to 6 be withdrawn.

For the foregoing reasons, all the claims now pending in the present application are believed to be clearly patentable

over the prior art of record. Accordingly, favorable reconsideration of the claims in light of the above remarks is courteously solicited. If the Examiner has any comments or suggestions that could place this application in even better form, the Examiner is requested to telephone the undersigned attorney at the below-listed number.

Respectfully submitted,

DATE: 25 February 2003



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Appendix

Amendments to the Claims

1. (amended) A method of detecting mutation in the base sequence of nucleic acid, including:

(A) a bonding step of hybridizing an object of analysis, consisting of nucleic acid or a nucleic acid fragment including a plurality of inspected sites to be subjected to inspection of mutation in the base sequence, with a plurality of [types of] labeled oligonucleotides of varying types, each oligonucleotide having a base sequence that is complementary to [any] at least one normal base sequence of one of the inspected sites [having normal base sequence], and each oligonucleotide being labeled to be discriminable from each other for forming duplexes including hetero- and homoduplexes; and

(B) a detection step of employing an ion pair chromatograph comprising a reversed phase column serving as a separation column and a detector capable of discriminating and detecting the labeled oligonucleotides, and setting the separation column at a temperature [causing the] at which there is a difference in stability between the hetero- and homoduplexes included in the duplexes for analyzing the object of analysis.

4. (amended) The mutation detecting method according to claim 1, which further comprises observing a chromatogram of labels obtained through the detection step (B), and thereby [for] determining that an inspected site corresponding to a label is non-mutational due to the presence of [having] a single peak [as non-mutational], while further determining that an inspected site corresponding to a label is mutational due to the presence of [having] two peaks [as mutational].